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PATENT & TRADEMARK OFFICE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT	:	SAMUEL ROSE, MD
SERIAL NO.	:	08/782,590
FILED	:	January 13, 1997
FOR	:	A METHOD AND COMPOSITION FOR TREATING CANCER BY CONVERTING SOLUBLE RADIOACTIVE TOXIC AGENTS INTO INSOLUBLE RADIOACTIVE TOXIC PRECIPITATES VIA THE ACTION OF NON-MAMMALIAN ENZYMES BOUND TO THE NON-ENDOCYTOSING RECEPTORS OF CANCER CELLS
EXAMINER	:	SUSAN UNGAR, PH.D.
GROUP ART UNIT	:	1640

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3-7-03

Commissioner of Patents and Trademarks
Washington, D.C. 20231

SIR:

1. I, George L. Mayers, hereby declare that I am a citizen of the United States and a resident of Gainesville, Florida.

2. I am currently retired Professor of Immunology from Roswell Park Cancer Institute. I have been in the field of cancer research for over 30 years, was former Chairman of the Department of Immunology at Roswell Park, and served as a Professor and Chairman of the Department of Immunology and Microbiology, Roswell Park Division, State

University of New York at Buffalo. Principally throughout my career I was a working research scientist in the field of cancer biology and cancer therapy.

3. I make this declaration under 37 C.F.R. 1.132 to traverse grounds of rejection of the above-identified U.S. Patent Application, Serial No. 08/782,590 of Samuel Rose, MD (hereinafter the '590 Application).

4. I have examined and am familiar with the original specification, claims, and drawing of the '590 Application; the Official Action mail March 18, 1998 (Paper No. 10); the Amendment filed September 21, 1998, the Official Action Mailed November 25, 1998 (Paper No. 18), the Official Advisory Action August 16, 1999, the Preliminary Amendment for Continued Prosecution Application November 16, 1999, the Official Action, May 25, 2000 (Paper No. 27), the Official Action, February 13, 2001 (Paper No. 33), The Continued Prosecution Application filed August 13, 2001, and the Official Action Mailed July 3, 2002 (Paper No. 40). I have also examined and am familiar with the excerpts from 2 confidential, unpublished reports, dated January 17, 1997 and June 20, 2000, prepared by Marin Biologic Laboratories, Inc. (a contract research laboratory) which provide in vitro scientific data supporting the present invention.

REGARDING OFFICIAL ACTION MAILED JULY 3, 2002 PAPER NUMBER 40 (HEREINAFTER "ACTION")

5. The present action refers back to objections from previous actions including Papers No. 33, 27, 18, and 10. I have made note of the objections of the previous actions in my responses below.

Paper 40, Page 3, line 17, (a')

" ... the arguments have been considered but not found persuasive (a') for the reasons previously set forth in Paper 15 ..."

Paper 18, Page 3-4, Line 17 (page 3) - Line 5 (page 4), (a')

" ... (a') a review of the cited support reveals general teachings of the chemistry of indoxyl chemistry but does not provide guidance on or exemplification of making or using the broadly claimed agents ...a review of the specification does not reveal the absolutely critical nature of radio-labeling of the therapeutic agent."

Paper 27, Section 4, Page 4, Lines 4-12, (a')

"... (a')General methods of indoxyl chemistry, preparation of precipitable material and methods of radiolabeling have been taught, but the specification does not provide guidance on or exemplification of making or using the broadly claimed agents that would be therapeutic in vivo and Applicant admits on the record that the claimed therapeutic agent is only therapeutic after conversion."

Response (a'):

To one of skill in the art, sufficient information is disclosed in the present invention to practice the said invention, including methods of making the broadly claimed first therapeutic agent and the radiolabeling of those agents for therapy.

The examiner agrees that sufficient detail for the preparation and radio-labeling of indoxyl-type first therapeutic agents is disclosed. In my opinion, the claims and specifications of the present invention also provide sufficient guidance to enable one of skill in the art to prepare and use the broadly claimed first therapeutic agent

The present invention discloses that cancer therapy using the claimed therapeutic agent is induced via the radioactive killing of the cells adjacent to the radioactivity that remains resident within the tumor for a long period (upwards of 5 to 7 days) and allows the products of radioactive decay to have their effect. The term therapeutic agent is meant to indicate this process as a result of converting the SPR from a soluble form to an insoluble form within the tumor which is carried out by the enzymatic activity of the bispecific agent attached to the non-endocytosing receptors of cancer cells or the extra-cellular matrix generated within the tumor such as CEA. The therapeutic agent claimed in the present invention is a radio-labeled soluble precipitating material for use as a pro-drug. Therefore, it is readily apparent to one of skill in the art that while circulating any toxicity

from the radioactivity is unwanted and not therapeutic but somewhat unavoidable, but the molecules are soluble, generally low molecular weight, so that if they are not transformed to a solid within the tumor, they are rapidly excreted through the kidneys before they do extensive damage to any normal tissues. Thus, the therapeutic agent disclosed in the present invention is only therapeutic when it is converted into a form that will keep it trapped within the tumor and provide the necessary time (5-10 days) for the radiation to have its effect on the tumor. The present invention teaches that conversion of the radio-labeled therapeutic agent into a radio-labeled precipitate in the extra-cellular fluid assures that it will remain within the tumor since the interstitial flow through the tumor is virtually absent because of the absence of or limited lymphatic drainage and the limited number of macrophages within the tumor.

The physiology of tumors including poor lymphatic drainage is common knowledge to those in the field of oncology. The radio-labeled SPR should be very stable and not undergo much degradation while circulating, but any that might occur should also be rapidly excreted by the kidneys. Based on the historic studies of Holt in 1950s (e.g. Nature 169, 1952 or J. Histochem. Cytochem., 4, 1956), the precipitate will form in extremely close proximity of the enzyme that is fixed to the first extracellular precipitate as he used these types of precipitates to define exact positions in histological sections.

Also disclosed in the present invention are compositions for other types of molecules that such a conversion from a soluble radioactive molecule to a radioactive precipitate could be achieved. For example, it is known to one of skill in the art that cellulose oligimers as a carbohydrate could be selected and that a decasaccharide is insoluble. Radio-labeling of such a composition is known to one of sill in the art. For example, it would be readily achievable to attach radio-iodo p-iodobenzoic acid as the ester to the cellulose oligimer to make it radioactive and then several galactose groups to form galactosides, the branching of which destroys the crystal structure which greatly increases the solubility and, thus, forms a radio-labeled SPR. Continuing this example, when introduced into the body, the therapeutic agent circulates to the tumor where and the enzyme moiety of the bispecific reagent bound to the non-endocytosing receptor of cancer cell, in this case a galactosidase, would cleave the galactosides from the radio-labeled decacellulose-galactoside$_{4\text{-}8}$ (i.e., having 4-8 galactosides attached to the decacellulose) and the

resulting radio-labeled-decacellulose molecules would precipitate in the tumor. One of skill in the art would recognize that any un-precipitated radio-label would be excreted in the urine.

The general example disclosed in the specification (p.23-24) provides adequate guidance to enable one of skill in the art to practiced the present invention without undue experimentation, For example, it is well-known that the other groups mentioned such a chitin, proteoglycans, synthetic polymers, and peptides in a similar fashion. In the case of peptides to fit use in this patent, they would have to be insoluble and relatively non-digestible such as the example of opio-melanins, or hair-like polypeptides, or silk-like polypeptides to which would be added the radio-label and solublilizing groups such glucoses, galactoses, glucuronic acid residues, etc. that can be cleaved by the appropriate enzyme (glucosidase, galactosidase, glucuronidase, etc.) moiety of the bispecific reagent previously bound to the non-endocytosing receptors of cancer cells to be used as a first therapeutic agent in the present invention. Such compositions could be used in the same way described for radio-labeled cellulose oligomers to provide a radioactive precipitate within the tumor for the requisite 5-10 days and thereby be therapeutic by killing the tumor cells.

Paper 40, Page 3, Line 18-19, (b')

"... (b') the arguments have been considered but not been found persuasive for the reasons previously set forth in Paper No. 27."

Paper 27, page 4, line 14-15, (b')

"... (b') Applicant is arguing limitations not present in the claims as currently constituted as immobilization of a radio-isotope is not claimed."

Paper 40, Page 3, Line 20-22, (c')

"...(c') Applicant is arguing limitations not recited in the claims as bispecific reagent is not being claimed, only a therapeutic agent being a soluble precipitable material is claimed."

Paper 27, Page 4, Line 14-17, (c')

"...(c') Applicant is claiming a therapeutic agent ... Applicant admits on the record that the claimed therapeutic agent is not therapeutic *per se.*"

Paper 40, Page 3, Line 22-23, (d')

"...(d') The arguments have been fully considered and are not persuasive for the reasons previously set forth."

Paper 40, Page 3, Line 23, (f')

"...(f') The arguments have been fully considered and are not persuasive for the reasons previously set forth in Paper 33."

Paper 33, Page 4, Line 12-15, (d')-(f')

"...(d')-(f') Applicant is arguing limitations not recited in the claims as presently constituted ... is not therapeutic."

Response (b'), (c'), (d'), (f'):

The specification and the claims enable the present invention to be practiced by one of skill in the art. The well-published literature of ADEPT (which teaches the conversion of a pro-drug into an active drug by the enzyme moiety of a previously bound bispecific reagent) provides a working example for the present invention where the radio-labeled therapeutic agent disclosed in the present invention is converted into an extra-cellular radio-labeled precipitate by the enzyme moiety of the previously bound bispecific reagent. The disclosure in the specification of the present invention enables one of skill in the art to predict with high expectation of success, and without undue experimentation, that the first therapeutic agent described in the instant invention will precipitate, will accumulate in the extra-cellular fluid and can and will function as described in the present invention.

On pages 9-10 of the present invention, Dr. Rose provides an overview of the field of ADEPT and many outstanding references that include suitable dose ranges for the bispecific reagent for different tumor systems. These examples provide sufficient guidance to one of skill in the art to predict the required dose ranges for the therapeutic

agent claimed in the present invention and to practice the invention without undue experimentation.

For example, based on the references for ADEPT cited in the present invention, one of skill in the art could make the following calculation and experimentation that shows that it is readily possible to provide enough binding for at lease 2 mg of a bispecific reagent (for example a monoclonal antibody galactosidase conjugate) to the non-edocytosing recptors of cancer cells. Using well-established data in the field of cancer research, it would be apparent to one of skill in the art that this is in great excess of the amount that could actually be used and that a fraction of these sites would be more than adequate to provide the radioactivity necessary for a successful therapy. (If the calculations for the presence of enough binding sites is required, I can prepare them for the examiner, but they didn't seem to fit with what was necessary to address the examiner's concerns and the studies with ADEPT certainly show that there should be enough binding sites at which to bind the bispecific reagent).

Assuming that we inject 100 mg of antibody galactosidase bispecific reagent into the rat, then based on observations for localization of antibodies, we can expect that only 0.01% of the injected dose is localized per gram of tumor or 10 ug bispecific reagent/g tumor Since 5/6ths of the molecule is the galactosidase, there is 8 ug galactosidase/g tumor.

8×10^{-3} mg/ml $\div$ 465000 mg/mmol $= 1.72 \times 10^{-8}$ mmol/ml or
a galactosidase concentration of 1.72×10^{-8} M in the extracellular space

According to David Goldenberg (Immunomedics, Inc.), 20 uCi of ^{131}I/g of tumor would exceed 1500 cGy which should effectively kill the tumor. In his publication [(J. Nucl. Med. 38: 391 (1997)], his data suggest that 100 uCi was much higher than 2000cGy in tumor with safe levels in the blood. He also showed that injection of 100 ug of Mab gave 20% of the injected dose/g tumor. Putting these facts together supports 20 uCi/g tumor, if 100 ug of Mab containing 100 uCi of ^{131}I were injected. A similar range can be gleaned from the data in Cancer Res. 55: 5842s (1995). .

$\lambda = 0.693/t_{½}$

where λ is the decay constant and $t_{1/2}$ is the isotope half life

$\lambda = 0.693/(8.1 \text{ days} \times 24 \text{ hr/day} \times 60 \text{ min/hr}) = 5.94 \times 10^{-5} \text{ min}^{-1}$

$$DPM = \lambda N$$

where DPM = disintegrations per minute and N = No. of atoms of radioactive isotope

$1 \text{ Ci} = 2.22 \times 10^{12}$ DPM; therefore 20 uCi = 4.44×10^{7} DPM

$4.44 \times 10^{7} \text{ DPM} = 5.94 \times 10^{-5} \text{ min}^{-1} \text{ N}$

$N = 7.47 \times 10^{11}$ atoms ^{131}I

7.47×10^{11} atoms/6.023×10^{23} atoms/mole = 1.24×10^{-12} moles of ^{131}I

This is the amount of radioactivity that is needed per gram of tumor, therefore 1.24×10^{-9} mmol/ml tumor, or a concentration of 1.24×10^{-9} M. Since the iodine will be attached to indoxylgalactoside, 1.24×10^{-9} mmol in a gram of tumor or 0.529 ng of ^{131}I-iodoindoxylgalactoside (MW 426.3) will have to be localized.

Based on Charles River data, the total extracellular fluid in a rat of 125 g is 35 ml.

Next, as the radio-labeled therapeutic agent disclosed in the present invention, let us assume that we could work with just the minimum amount of ^{131}I-iodoindoxyl-galactoside required to get 20 uCi per gram of tumor or 1.24×10^{-9}M. Since this concentration is $1/10^{th}$ of the galactosidase concentration in the extracellular space noted above, to one of skill in the art it is possible to use bi-molecular enzyme kinetics for evaluating the rate of conversion. Since the tumor concentration is in equilibrium with the large circulatory concentration in the 35 ml of ECF, steady state conditions should be rapidly established and maintained with respect to the substrate concentration.

Therefore using Michaelis-Menten Equation for the enzyme reaction below (Abeles, Frey and Jencks *Biochemistry*, Jones and Bartlett Publishers, 1992):

$$E + S \underset{k_{-1}}{\overset{k_1}{\leftrightarrow}} ES \xrightarrow{k_2} E + P$$

$$v = \frac{k_2[E]_{tot}}{\frac{k_2/k_1}{[S]} + 1}$$

and since k_{-1} should be<<< k_2, $k_{cat} = k_2$ and $k_{cat}/K_m = k_1$, it is possible to use the constants for E. coli β-Galactosidase for p-nitrophenylgalactoside from Roth and Huber, J. Biol. Chem. 271:14296-14301 (1996) $k_{cat} = 90\ s^{-1}$, $k_{cat}/K_m = 2200\ s^{-1}mM^{-1}$, $K_m = 0.041$ mM.

Thus:

$$v = \frac{90\ sec^{-1}\ [1.72 \times 10^{-8}M]}{(90\ sec^{-1})/(2200\ sec^{-1}\ mM^{-1})/(1.24 \times 10^{-6}\ mM) + 1}$$

$$v = 4.69 \times 10^{-11}\ M\ sec^{-1}$$

In this example, we are talking about 1 gram of tumor or ~ 1 ml, we are making 4.69×10^{-11} mmole per sec. From the previous calculations, we need a total of 1.24×10^{-9} mmoles of ^{131}I per gram of tumor. Dividing the latter by the former suggests that the enzyme should make the necessary 20 uCi in 26sec. Since it only requires the injection of 0.7 mCi [20uCi/g tumor X 1g tumor/ml tumor X 35 ml ECF], and since most of the low MW substrate should be rapidly excreted, there doesn't seem much likelihood of general cytotoxicity to the normal tissues.

Further, one of skill in the art would eb able to extrapolate that we use 100000 times as much iodoindoxylgalactoside or 1.24×10^{-4}M. Even with this great increase we are in a range where we need the bimolecular equation since the rate is still below the V_{max} for the enzyme. At this point we would be injecting 1.85 mg of iodoindoxylgalactoside into the rat (1.24×10^{-4} mmol/ml X 35 ml X 426.3 mg/mmol). Using the equation for enzyme kinetics above:

$$v = \frac{90\ sec^{-1}\ [1.72 \times 10^{-8} M]}{(90\ sec^{-1})/(2200\ sec^{-1}\ mM^{-1})/(1.24 \times 10^{-1}\ mM) + 1}$$

$$v = 1.16 \times 10^{-6}\ M\ sec^{-1}$$

Therefore, the enzyme is converting 1.16×10^{-6} mmoles of iodoindoxylgalactoside per sec (or 4.96×10^{-4} mg of iodoindoxylgalactoside per sec). Thus, the invention disclosed in this application needs 1.24×10^{-4} mmoles/g tumor if the radioiodine is only 0.001% of the sample. This amounts to 106.5 sec, which should deposit 31.9 ug of iodoindigo (MW 514.1) in the gram of tumor. This should be in the range that will form a precipitate, since I isolated from cells in culture, using Solvable to digest the cells followed by centrifugation to collect the indigo precipitate, 64 ug of bromoindigo.

The present invention allows for an injected dose of even 100 times that level of iodoindoxylgalactoside or 1.24×10^{-2}M and not be toxic. This would put the sytem in the V_{max} range (where the denominator of the equation approaches 1). It would thus be necessary to inject into the rat 185 mg of iodoindoxylgalactoside of which 0.00001% of the sample would contain radioiodine. We use the equation to determine rate of deposit in the tumor: $V_{max} = k_2\ [E]_{tot}$.

Thus:

$$V_{max} = 90\ sec^{-1}\ [1.72 \times 10^{-8}\ M] = 1.548 \times 10^{-6}\ M\ sec^{-1}$$

The present invention would thus require1.24×10^{-2} mmol of iodoindoxylgalactoside per gram of tumor tissue, it will take 8010 sec or 2.22 hrs to get the indigo precipitate of 3.19 mg of iodoindigo/g tumor tissue.

These calculations show that under a number of different scenarios for indoxyl's, enough radioactivity can be delivered to the tumor to be therapeutic. Similar results would be expected for any of the other potential reagents such as the appropriate carbohydrates, peptides, synthetic polymers, etc. Thus, these reagents only become therapeutic when converted to a precipitate and remain in place to deliver the radioactive dose to the tumor cells.

Paper 40, Page 4, Line 1-3, (e'), (g'), (h')

"...(e'), (g'), (h') the arguments are not persuasive for the reasons previously set forth in Papers Nos. 15 and 27."

Paper 27, Page 4-5, Line 19-23 (p. 4) – Line 2 (p.5), (e')

"...(e') it is clear that the limitation that the therapeutic agent ... other than claim 83, none of the claims are drawn to a radio-label soluble precipitable material and none of the claims are drawn to immobilized reagents ...not predict that the only location where the therapeutic agent will be immobilized will be at the site of the bispecific reagent."

Paper 18, Page 4, Line 19-22, (e')

"... (e') ... the therapeutic agents may be inactivated *in vivo* ... rejection is maintained."

Paper 10, Section 5, Page 6, Lines 11-17

"...(e') The therapeutic agents may be inactivated *in vivo* before producing a therapeutic effect, for example, by proteolytic degradation, immunological activation or due to an inherently short half-life."

Response (e'):

There are at least two well defined systems described in previous literature that would make one skilled in the art predict that the therapeutic agent would form in the tumor extracellular space. Based on ADEPT, we know that conversion of pro-drug to active-drug is only via the enzyme with specificity for the substrate provided and one of skill in the art would expect the first therapeutic agent disclosed in the present invention to behave the same way. Second, based on the studies of Holt and others doing histochemistry, where the enzyme converts the substrate to a product that is a precipitate, as in this patent application, the precipitate will form in extremely close proximity to the enzyme, usually close enough that it can be used as a marker for the physiological structures to be identified at a microscopic level with great accuracy. This level of precision is certainly not required for this therapy, but does suggest how defined the

precipitate would be around the enzyme molecules that are fixed to the first extracellular precipitate throughout the tumor.

The question of inactivating the therapeutic reagent in vivo prior to its having its effect is a little difficult answer since there is no therapeutic effect until it is precipitated in the tumor. Activity from the radio-isotope prior to the formation of the precipitate that occurs in the circulation is actually detrimental to the host. I think that the examiner is comparing this to a normal pharmaceutical drug that can be deactivated. Since the pharmacological activity is due to the radioactivity, it can only be deactivated by removing the molecule or at least the radio-isotope. The structure has been selected to be stable under physiological conditions (i.e., the isotope should remained attached to the substrate) and any early conversion to a precipitate in normal tissues should expedite the clearance through the macrophages present in the normal tissues. Since a large percentage of the injected material (as when radio-iodide is used in the therapy of thyroid cancer) is expected to be excreted, the loss of a small amount by early conversion should not hamper this therapeutic approach in any way.

Paper 40, Page 4, Line 1-3, (g')

"...(e'), (g'), (h') the arguments are not persuasive for the reasons previously set forth in Papers Nos. 15 and 27."

Paper 27, page 5, line 6-10, (g')

"... (g') Applicant was invited to submit objective evidence to resolve this issue, no objective evidence has been submitted but Applicant has admitted on the record that 'ultimately, the insoluble precipitate will be removed by convection and phagocytosis but such removal from tumor tissue will be slower than for normal tissues."

Paper 18, Section 5, Page 5, Lines 10-15, (g')

".. (g') Applicant's stated opinion is noted but it is clear that one of skill in the art would expect that an insoluble precipitate would be removed from the claimed region either by convection, diffusion or by phagocytosis."

Response (g'):

I strongly concur with the statement of Dr. Alan Epstein that lymphatic drainage of tumors is very poor and the presence of macrophages within the tumor is very low. I would also note that there are many publications in which R. Jain (Harvard University) has reported the absence of lymphatic drainage and many of his studies on internal tumor pressures are based on the absence of these lymphatics.

As discussed in the Declaration of Dr. Alan Epstein submitted previously and in the specification of the '590 Application, published research in the field of tumor biology describes how tumors are known to have very low flow rates (some of these references have been cited in the specification of the present invention on pages 35-36, in Exhibits B and C submitted with the response filed May 25, 1999, some have been cited by the Examiner, and others are well-known in the field). Further, it is known that in developed tumors, there is very dense packing of tumor cells. Thus, even in the absence of data, one of skill in the art would predict that the precipitate formed from the first therapeutic agent in the extracellular space of the tumor will remain trapped between the cells and that the precipitate would be removed very slowly. This is also supported by the Marin Biologic data on the beads because examination of the beads showed trapped indigo precipitate caught in the matricies of the beads. In addition, as described in the specification (page 35-36) and as known to one of skill in the art, not only do tumors have a very slow flow rate compared to normal tissue, but tumors also lack macrophages, lymphocytes, and monocytes and thus precipitate formed in the extracellular space of the tumor would not be removed as quickly as from normal tissues where macrophages, monocytes and lymphocytes are active and rapidly remove unwanted particles. Many histological studies of tumors have shown large numbers of macrophages and lymphocytes in the tissue volume surrounding a tumor, but extremely few, if any, within the tumor volume. This is an observation that has not been explained. However, when tumors undergo remission, macrophages do enter the necrotic areas and help in the clean up. Oncologists as far back as the 1970s have tried to activate the macrophages surrounding the tumors as a form of therapy without much success. Based on the slower, tortured flow rates known to occur in tumors and the absence of macrophages and lymphocytes within the tumor to

help remove insoluble material, one of skill in the art could reliably predict that the rate of removal of precipitate from the tumor would be very much slower from tumor tissue compared to normal tissue.

6. I hereby declare that all statements made herein of my own knowledge and are true and that all statements made on the information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine, imprisonment, or both under Section 1001 of title 18 of the United States Code.

Date: 2 Jun 2003

Signed: